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(71) Applicant(s)
Jacques Protic
Bryn Du, Llanbedrgoch, Isle of Anglesey, LL76 8NX, United Kingdom

(72) Inventor(s)
Jacques Protic

(74) Agent and/or Address for Service
Potts, Kerr & Co
15 Hamilton Square, BIRKENHEAD, Merseyside, CH41 6BR, United Kingdom

(54) Abstract Title
Sterilisation process

(57) Sterilisation is carried out without using a vacuum. The sterilisation chamber or area to be sterilised is subjected to an aldehyde in a gaseous state, then gas plasma is introduced or generated within the chamber. The aldehyde may be formalin. Ozone may also be added to increase the sterilisation effect and to remove any residual formalin. The process provides improved sterilisation of medical apparatus.

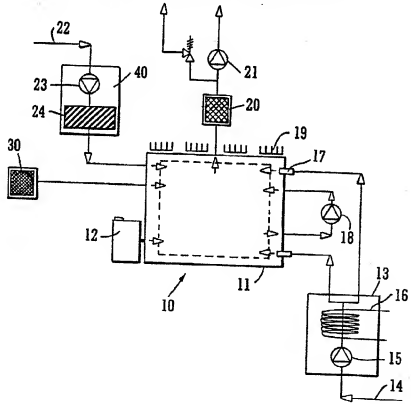

FIG. 1

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At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

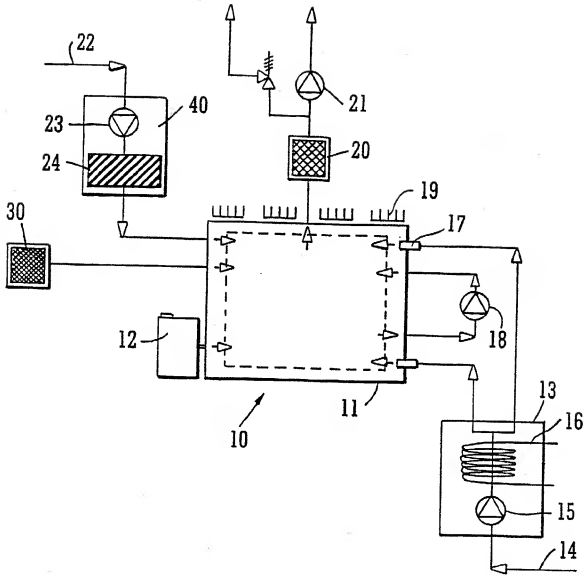


FIG. 1

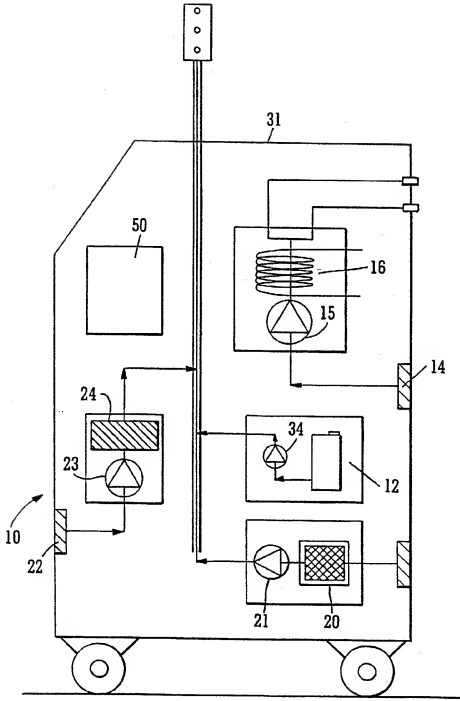


FIG. 2

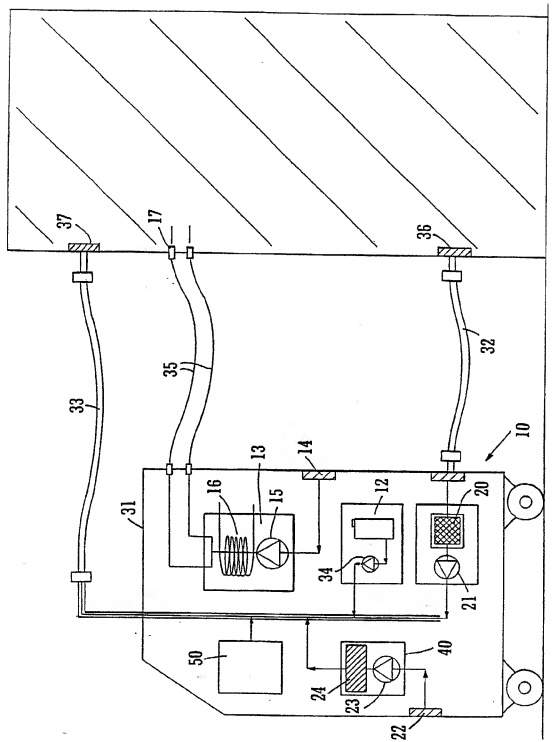


FIG.3

Figure 4. **Sterility Assurance Level (SAL) graph for *B. stearothermophilus* derived from unwrapped (nude) 10^6 spores on stainless steel carriers**

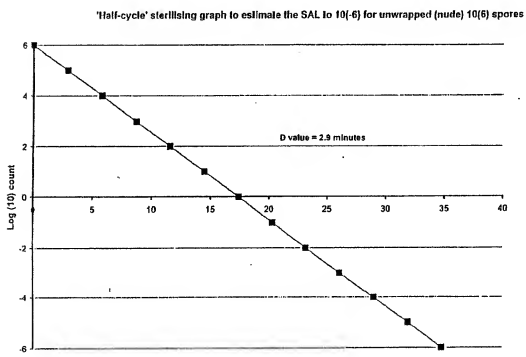


Figure 5. Sterility Assurance Level (SAL) graph for *B. stearothermophilus* spores derived from 10^6 spores on stainless steel carriers wrapped and sealed in Tyvek(R) sterilising envelopes

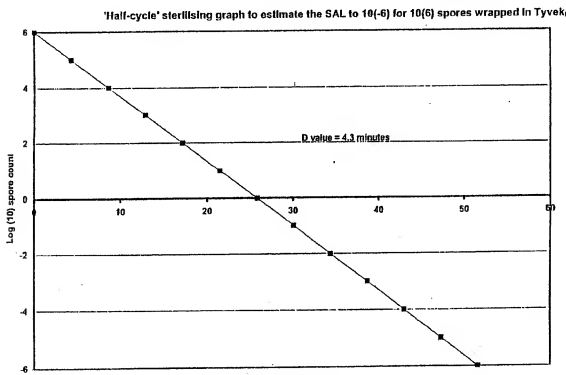
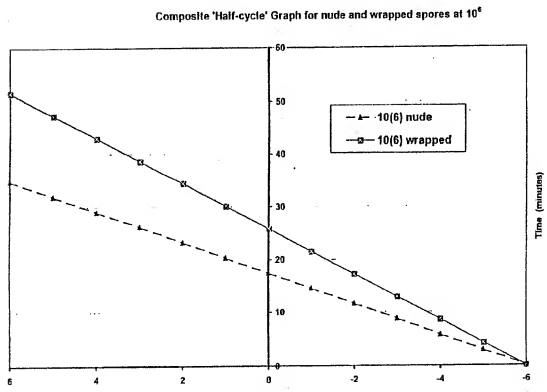


Figure 6. **Composite graph to estimate the SAL to 10^{-6} for unwrapped (nude) and wrapped (Tyvek$^{(R)}$) spores of *B. stearothermophilus* at 10^6**



A STERILISATION PROCESS AND APPARATUS THEREFOR

The present invention relates to a sterilisation process. In particular, the present invention relates to a sterilisation process that does not need to be carried out in a vacuum. Additionally, the present invention relates to an apparatus therefor.

As will be appreciated, it is imperative that materials and devices used for the practice of medicine are aseptic.

As a result thereof, much research has gone into the development of processes suitable for effecting sterilisation of such materials and devices.

Generally, such processes are carried out by the manufacturer of such materials and devices, or by users thereof, for example, by medical establishments, such as hospitals.

One sterilisation process currently being utilised involves exposing such materials and devices to ethylene oxide gas for at least one hour and then leaving same to aerate for a minimum period of twelve hours. As is well documented, one of the major problems associated with the use of ethylene oxide gas is that it is highly toxic and dangerous to humans, that is, it is a known carcinogen and mutagen. Additionally, and due to the aeration time required, it is time consuming.

Another low temperature sterilisation process involves treating, by irradiation, the materials and devices with Gamma radiation. Although such process overcomes the disadvantages associated with the use of ethylene oxide, it will be appreciated that the apparatus therefor is extremely expensive and more importantly, it cannot be used to sterilise any electro medical

devices or items, since any item including a memory chip is simply wiped clean by the action of Gamma radiation thereon.

Another sterilisation process involves the use of steam autoclaving. As will be appreciated by those skilled in the art, one of the problems associated with the use of such a process is that it requires high temperatures and therefore, is not suitable for sterilising materials or devices made out of matter that is affected by either moisture or high temperature, i.e. its application is fairly limited and depends on the inherent nature of the matter out of which the material or device to be sterilised is made.

With a view to overcoming the problems associated with the sterilisation processes outlined above, a number of low temperature sterilisation processes, involving the use of gas plasma, have been developed.

One of the first sterilisation systems involving the use of gas plasma that received approval from the Food and Drug Administration (FDA) was the STERAD sterilisation system manufactured by Advanced Sterilisation Products, a division of Johnson and Johnson. Such system operates as follows:

Items to be sterilised are placed into the sterilisation chamber of the STERAD steriliser and air is evacuated therefrom to produce a vacuum. When a sufficiently low pressure is achieved within the chamber, a low temperature gas plasma is generated to aid with the removal of any residual moisture from the items being sterilised. This is known as the pre-plasma stage.

At the end of the pre-plasma stage, the system is vented to atmospheric pressure by the introduction of filtered air. This process step constitutes the end of the pre-treatment drying phase, and the sterilisation process then begins.

To start sterilisation, the pressure within the chamber is reduced and an aqueous solution of hydrogen peroxide is injected into and vaporized within the chamber. The resulting hydrogen peroxide vapour diffuses through the chamber thereby surrounding the items to be sterilised and initiating the inactivation of the microorganisms.

The pressure within the chamber is then increased, and then following a subsequent pressure reduction, a low temperature plasma is generated by applying RF energy to create an electric field that in turn initiates the generation of the plasma. In the plasma, the hydrogen peroxide vapour is broken apart into reactor species known as free radicals. After the activated components react with the organisms, other materials, or each other, they lose their high energy and recombine to form oxygen, water vapour and other non-toxic by-products. This constitutes one half of the total sterilisation process, which is then completed by repeating the above steps, that is, with the exception of repeating the pre-treatment drying phase.

At the completion of the second half cycle, the RF energy is turned off, the vacuum is released, and the chamber is returned to atmospheric pressure by the introduction of HEPA-filtered air.

Although widely used, the STERAD apparatus and process exhibits the following disadvantages:

A. Moisture interferes with the STERAD steriliser's ability to attain vacuum conditions. As a result thereof, the presence of excess moisture will result in the STERAD apparatus aborting the sterilising process being carried out therein;

B. Sterilisation will not occur if any organic material is present, since the sterilising agent will decompose. This results in the necessity of ensuring that the items to be sterilised are thoroughly clean before being placed in the STERAD steriliser. As will be appreciated, this is time consuming;

C. The STERAD steriliser cannot sterilise items having long (greater than 12 inches) or narrow lumens without the assistance of a "diffusion intensifier". As will be appreciated, such a constraint limits the STERAD steriliser's utility;

D. The items to be sterilised can only be packaged, or wrapped, in polypropylene sterilisation wrap or polypropylene pouches. In this connection, the STERAD system cannot achieve sterilisation of items wrapped in tear-proof paper, which is the standard packaging material used extensively in hospitals. The reason being is that the hydrogen peroxide, which is in droplet form, would be absorbed by the tear proof paper wrapping thereby making the STERAD process ineffective. As a result thereof, it is recommended that users of the STERAD steriliser use a special and considerably more expensive synthetic wrapping made by Du Pont and sold under the trade mark, Tyvek ®;

E. As with other sterilisation processes involving the use of hydrogen peroxide, liquids, powders and absorptive materials, such as paper, cannot be sterilised due to their tendency to absorb hydrogen peroxide. As will be appreciated, such absorption hinders sterilisation efficiency and aeration of the sterilant;

F. As a vacuum is required to carry out the STERAD process, the size of the sterilising chamber is limited. As will be appreciated, this makes the STERAD steriliser suitable for relatively small volume process applications only; and

G. It has been documented that hydrogen peroxide is not as penetrating as ethylene oxide.

According to the present invention, there is provided a sterilisation process which is not carried out in a vacuum including the steps of:

introducing an aldehyde in a gaseous state into a sterilisation chamber or area to be sterilised; and

introducing gas plasma into the sterilisation chamber or area to be sterilised.

It is believed that the sterilisation process of the present invention at least addresses some of the disadvantages associated with the STERAD process outlined above. In particular, and since the process of the present invention is not carried out in a vacuum, the presence of small traces of moisture does not inhibit sterilisation. Moreover, as the sterilisation process of the present invention does not need to be carried out in a vacuum, it can be utilized for large volume applications, for example, it can be utilised to sterilise laboratory rooms, bio-hazardous areas and similar environments or enclosures. Furthermore, the process of the present application is still effective even when the materials or devices to be sterilised are soiled. In this connection, and during our investigations, we smeared items to be sterilised with quick drying protein and observed that same had no effect on the

effectiveness of the process of the present invention. Consequently, it is believed that the process of the present invention is more penetrating and moreover, does not require a pre-sterilising check, which is time consuming, to ensure that the items to be sterilised are thoroughly clean.

In a preferred embodiment, the aldehyde is formalin, that is, formaldehyde in gaseous state. Further preferably, the sterilisation chamber area to be sterilised is provided with formalin at a concentration of 2000 – 6000 parts of a million (ppm), further preferably, 3000 ppm. One of the main advantages of using formalin is that same can be utilised with both standard paper wrapping and synthetic specialised wrappings like Tyvek ®, since formaldehyde is readily evaporated by exposure to elevated temperatures.

When an aldehyde, like formalin, is exposed to high-energy gas plasma it is believed that H-C=O. radicals form, which will react with oxygen to form a radical intermediate. This in turn will react with formaldehyde to produce performic acid and ultimately, formic acid.

$H\text{-}C{=}O\cdot + O_2 \rightarrow H\text{-}C{=}O.O\cdot + H_2C{=}O \rightarrow HC{=}O.OOH$ (performic acid) $\rightarrow$ $H\text{-}C{=}O.OH$ (formic acid)

The gas plasma is a partially ionized gas composed of ions, electrons, and neutral species. Such a state of matter can be produced through the action of either very high temperatures or strong electric or magnetic fields. Preferably, the ionised gas is produced by gaseous electric discharges from dry compressed air at a constant pressure of 6 bar and continuous airflow at 10 liters per minute. Preferably, the pressure inside the sterilization chamber or area to be sterilized during the gas plasma phase is lower and is controlled to 1 – 100, preferably 10, pascals above atmospheric.

In an electrical discharge, free electrons gain energy from the imposed electric field and lose this energy through collisions with neutral gas molecules. The energy transfer process leads to the formation of a variety of highly reactive products including meta-stable atoms, free radicals, and ions. For instance, in an oxidizing plasma some of the active species are the atomic oxygen and '.DELTA.g molecular oxygen which is also called "singlet oxygen". A singlet molecule is one in which the absorption of energy has shifted a valence electron from its normal bonding orbital to an antibonding orbital of higher energy, and in which the electron spins are paired (oxygen is an unusual diatomic molecule in that the spins of the two valence electrons of lowest energy are not paired in the most stable or ground state). The resultant excited molecule is highly unstable and must release its excess energy through different pathways or re-combinations. Artificially generated singlet oxygen reacts with regions of high electron density in microbial substrates. For an ionized gas produced in an electrical discharge to be properly termed a plasma, it must satisfy the requirement that the concentrations of positive and negative charge carriers are approximately equal.

It has been found that small amounts of vaporised formaldehyde (formalin) and free radicals present in the low temperature gas plasma greatly increases the overall biocidal action of the gas plasma, particularly if the required conditions are generated under a positive pressure regime.

Due to the presence of atomic or excited oxygen in the gas phase, the formalin can produce very reactive short-life epoxides which can interact with many proteins and nucleic acids groups in the outer layer coats of spores to enhance sporicidal activity. This action is also highly effective against non-sporulated bacteria.

The sporicidal activity of formalin seems directly related to the concentration of free aldehyde radicals. In addition, the oxidation of formaldehyde gas into formic acid (HCOOH) as a vapour increases the sporicidal efficacy of epoxides. In the case of formalin, there is a very favourable set of conditions to attack the spore outer layers with active oxygen and facilitate several types of reactions with proteins, while also increasing the penetration and density of formaldehyde radicals in the critical areas of the spore. As such, a low temperature oxidizing gas plasma seeded with formalin will provide numerous reactive intermediates and free radicals to alter the spore outer coats and thus improve the diffusion of bactericidal groups. This mechanism explains why short exposures to a plasma gas in the presence of formaldehyde (formalin) can quickly destroy spores of their germinating capabilities. While excited ions, gas molecules and photons profoundly modify the protective layers of the spores, the active formaldehyde radicals effect the cell structures and initiate many additional lethal reactions which accelerate the killing process.

It is to be understood that within the context of the present invention the introduction of gas plasma into the sterilisation chamber or area to be sterilised includes generating the gas plasma within the sterilisation chamber or area to be sterilised and/or introducing the gas plasma into the sterilisation chamber or area to be sterilised from an outside source. It is also to be understood that the gas plasma can be generated from air and/or oxygen and/or an aldehyde in a gaseous state.

In a preferred embodiment, and subsequent to the introduction of plasma, ozone is introduced into the sterilisation chamber or area to be sterilised. The advantage of adding ozone is twofold. Firstly, and as an oxidising agent, it is highly bactericidal and thus enhances sterilisation, and

secondly, it can neutralise any harmful residue remaining within the sterilisation chamber or area to be sterilised, that is, once sterilisation is complete. For example, and when formalin is introduced into the sterilisation chamber or area to be sterilised, ozone will convert any formalin gas residue into carbon dioxide and water. Further preferably, and in addition to, or instead of, the addition of ozone, the residue may be neutralised by passing same through a carbon filter. In this connection, it is preferable that the sterilisation chamber or area to be sterilised is flushed with air drawn through a ULPA or HEPA filter.

In a preferred embodiment, prior to introducing gas plasma into the sterilisation chamber or area to be sterilised, the gaseous aldehyde is re-circulated within the sterilisation chamber or area to be sterilised for a period of 5 to 15 minutes, preferably 10 minutes. It is believed that such re-circulation provides optimum initial exposure of the aldehyde to the microorganisms to be eradicated.

Further preferably, the plasma is produced or generated for 15 – 180 minutes, preferably 45 minutes. It is believed that such duration is sufficient to enable the required interaction between the free radicals produced within the plasma field and the vital cell components, such as cell membranes, enzymes and nucleic acids, such that the life functions of the organisms to be eradicated is disrupted.

Further preferably, during the plasma producing stage, gas within the sterilisation chamber or area to be sterilised is re-circulated to give maximum exposure. It is to be understood that during such re-circulation the gas can be passed or re-circulated through the gas plasma generating means, for example, a gas plasma generator.

Further preferably, and during the production of plasma, additional oxygen electrons or free radicals are introduced into the sterilisation chamber or area to be sterilised. Preferably, this is effected by drawing air and/or oxygen into the sterilisation chamber or area to be sterilised, preferably through the gas plasma generator.

In a preferred embodiment, during the production of plasma, the temperature within the sterilisation chamber or area to be sterilised is maintained from 25 °C to 66 °C, preferably at 50 °C or within +/-3 °C thereof. By varying the temperature, it will be appreciated that the internal pressure within the sterilisation chamber or area to be sterilised will correspondingly change thereby enabling better penetration of items that need to be sterilised; even items which are wrapped.

In a preferred embodiment, the temperature within the sterilisation chamber or area to be sterilised prior to the production of plasma is maintained from 22 °C to 45 °C. During our investigations we noted that an increase of temperature prior to the introduction of the gaseous sterilising agent, in particular, formalin, improved the effectiveness of same. More particularly, we observed that the effectiveness of formalin was increased when the sterilisation chamber or area to be sterilised was maintained at a temperature of 45°C.

In a further aspect of the present invention there is provided a sterilisation apparatus when used to carry out the sterilisation process in accordance with the present invention, the sterilisation apparatus being provided with a sterilisation chamber or being connectable to an area to be sterilised and including:

means for providing the sterilisation chamber or area to be sterilised with an aldehyde in a gaseous state; and

means for introducing or generating plasma within the sterilisation chamber or area to be sterilised.

In a preferred embodiment the plasma generating means are isolated such that they cannot come into contact with the items to be sterilised. This has the advantage in that a sterilisation apparatus in accordance with the present invention is less likely to short circuit. That is, and as will be appreciated by those skilled in the art, since the plasma generating electrodes of the Sterad apparatus are an integral part of the sterilisation chamber's inner walls, same is more prone to short circuiting due to the metal items to be sterilised coming into contact therewith. This being the reason why the proprietors of the Sterad apparatus state that any metal items must be kept from coming into contact with the sterilisation chamber's inner or internal walls.

The following non-limiting embodiments of a sterilising apparatus in accordance with the present invention are given by way of example, and with reference to, the accompanying drawings in which:

Figure 1 is a cross-sectional view of a first embodiment of a sterilisation apparatus in accordance with the present invention;

Figure 2 is a cross-sectional view of a second portable sterilisation apparatus in accordance with the present invention; and

Figure 3 is a cross-sectional view of the sterilisation apparatus of Figure 2 when being utilised to sterilise a room.

As illustrated in Figure 1, a sterilisation apparatus 10 in accordance with the present invention includes a sterilisation chamber 11 into which the items to be sterilised can be located. During our investigations (see below), the sterilisation chamber 11 had a volume of 60 litres.

A chemical dosing unit 12 is in communication with the sterilisation chamber 11. In use, the chemical dosing unit 12 dispenses the chosen aldehyde in a gaseous state thereinto. In this connection, the dosing unit 12 includes a heating element for vaporising the aldehyde such that same can be introduced into the sterilisation chamber 11 as a gas. Preferably, the heating element is automatically inactivated once all the aldehyde has been vaporised.

Within the sterilisation chamber 11 there are two plasma electrodes 17 that are attached to a plasma generator 13, which includes an air inlet 14, an air pump or compressor 15 for drawing air into the plasma generator 13 and a HF transformer 16. It is to be understood that the number of plasma electrodes 17 may be varied depending on the volume of the sterilisation chamber or area to be sterilised, for example, if the sterilisation chamber or area to be sterilised is large, then there may be 4-6 plasma electrodes. Preferably, the electrodes 17 enter the sterilisation chamber 11 via isolated and sealed apertures. Preferably, the compressor 15 delivers air to the HF transformer 16 at a pressure of 5.5 bar and flow rate of 12 litres per minute.

The sterilisation chamber 11 is further provided with, or connectable to, a re-circulation system 18 for pumping or re-circulating the air within the sterilisation chamber 11.

The sterilisation chamber 11 is further associated with heat absorption cooling modules 19, which, in use, are used to cool the sterilisation chamber 11 such that the temperature within the chamber 11 can be maintained at a desired level.

An ozone generator 40 is also associated with the sterilisation chamber 11. Such generator 40 includes an air inlet 22, an air pump 23 and an ozone unit 24. In use, the air pump 23 draws dry air into the generator 40 via the air inlet 22 and pumps the air through the ozone unit 24 and into the sterilisation chamber 11. In this connection, it is to be understood that the dry air can be drawn directly from a supply of pure oxygen, for example, from a canister of oxygen that is connected to the air inlet 22.

The sterilisation chamber 11 is further associated with an extraction system including an activated carbon filter 20 and an air pump 21. In use, and after the generation of plasma, any harmful residue can be neutralised by extraction through the carbon filter 20. In this connection, and so that the sterilisation chamber can be flushed with clean air, same is connected to an ULPA filter 30. It is to be understood that a HEPA filter can be used instead of, or in addition to, the ULPA filter 30.

In order to test the efficacy of the sterilisation process of the present invention against bacterial spores, the sterilisation chamber 11 was loaded in accordance with ISO 11138, BS EN 866, EN ISO 14937 (draft), BS EN 1174, ISO 14161 (draft) with *Bacillus stearothermophilus* spores prepared on stainless steel carriers having a count of 10^6.

Formalin gas at a concentration of 3000 ppm was then introduced from the chemical dosing unit 12 into the sterilisation chamber 11.

The temperature, which was previously ambient, was increased from 22 °C to 45 °C, and the formalin gas was re-circulated via the re-circulation system 18 for 15 minutes within the chamber 11.

The plasma generator 13 was then activated and the gas plasma produced thereby was introduced into the sterilisation chamber such that the gas plasma and formalin could be re-circulated within the sterilisation chamber for the designated time periods tabulated in Table 1 below. During plasma generation, the temperature within the sterilisation chamber 11 was increased to 50 °C and maintained to within +/-3°C thereof by the heat absorption cooling modules 19.

Once the plasma generator 13 was inactivated, the ozone generator 40 was activated for approximately 15 minutes introducing ozone at a rate of 1.7 grams per hour into the sterilisation chamber 11.

Finally, the air pump 21 of the extraction system was activated thereby drawing clean air into the sterilisation chamber 11 via the ULPA filter 30 and discharging the neutralised residue back into the lab via an activated carbon filter 20. Such extraction and neutralisation lasted for 5 minutes.

The sterilisation chamber's door was then opened and each carrier was removed individually with sterile forceps and placed immediately into a separate bottle of Tryptic Soy Broth (TSB). As will be appreciated, TSB is an effective recovery medium, which includes amino acids such as tryptophan for neutralising any residual formaldehyde within a partially surviving cell.

The bottles were then incubated in a water bath for 5 days at 56°C and readings were taken from them and tabulated as shown in Table 1 below. In this connection, if the contents of a bottle was clear then the sterilisation process was taken to be a success and if the contents of a bottle were turbid, then the sterilisation process was taken to have failed. As part of the control, unexposed steel carriers were included with each batch to be tested.

In addition, the spore carriers were tested either unwrapped ('nude') and hence, directly exposed to the formalin vapour-gas plasma sterilisation process, or wrapped and sealed. In this connection, wrapping material included either special Tyvek® sterilising bags (base material manufactured by DuPont Products S.A.) with Tyvek® sterilisation envelopes or paper sterilisation envelopes obtained from Westfield Medical Ltd.

Results gained with the 10^6 spores are given in Table 1 and Figures 4-6.

The method utilised by us was validated by placing 9 spore carriers in the chamber 11 without any gas plasma being produced. It was found that all 9 spore carriers grew at day 1 in TSB (Table 1). Thereafter, one spore carrier that had not been exposed to gas sterilization was included with each batch of spore carriers placed in gas sterilization and it was observed that the unexposed spore carriers were always found to yield growth within 24 hours incubation at 56°C in the water bath.

As regards the manner in which the 'D' values (rate of kill for each logarithm_{10} count of spores) were calculated, we utilised the formulas for fraction negative methods (International Standard (ISO) 11138-1,1994; British Standard BS EN 1174-1: 1996; British Standard BS EN 866-1,1997 and Draft BS EN ISO 14161 Document 97/125276). In addition, the Holcomb-Spearman-Karber procedure was used for unwrapped 'nude' spore carriers

(Table 1) when non-constant numbers of samples were tested and the limited Spearman-Karber procedure was used for wrapped 10^5 spores (Table 1) when testing was carried out in batches of 8.

The D value (the time to kill 90% of organisms) for 10^6 *Bacillus stearothermophilus* spores on "nude" stainless steel carriers was 2.9 minutes, and was 4.3 minutes for those wrapped and sealed in Tyvek® envelopes.

As will be appreciated, a sterilising process should achieve at least a 10^{-6} Sterility Assurance Level (SAL) to be sure that the chance of a surviving organism is less than 1 in 10^6 replicates exposed in the sterilisation chamber at the same time. In practice, this is achieved by constructing a graphical presentation of the Half-Cycle method of steriliser validation for 'nude' spores (see Figure 4) and for wrapped spores (see Figure 5) with a composite graph (see Figure 6). The half-cycle time is illustrated for an exposure of 10^6 spores based on the D values calculated above. The full cycle time is then taken as twice the half-cycle time giving a SAL of 10^{-6}, that is, of course, assuming that a spore count not greater than 10^6 is placed in the sterilisation load in the sterilisation chamber 11. If a higher count of spores than this figure is present, then the sterilising cycle time needs to be extended accordingly. If the microbial and spore load is less than 10^6 then the sterilising time can be reduced. It is thus useful to know the approximate bacterial count expected on items to be sterilised in order to calculate an appropriate time to achieve a SAL of 10^{-6}.

As will be appreciated, our results show that the gas plasma process has penetrated very effectively through the Tyvek® plastic wrapping film to sterilise the spores contained within it. In addition, our investigations have shown that the gas plasma process of the present invention penetrates effectively through paper envelopes manufactured for medical sterilisation.

In addition, reliability has been demonstrated by repeated experiments demonstrating zero growth out of 8 replicates (Fraction Test Method) after 25, 30, 35 and 40 minutes of testing with gas plasma for nude (unwrapped) spore carriers at 10^6 *B. stearothermophilus* and after 35, 40, 45 and 60 minutes for wrapped spore carriers (Table 1). Reliability has also been demonstrated with the vegetable bacteria. (see below)

Table 1
RESULTS OF BACTERIAL SPORE TESTS with B. *stearothermophilus* spores on stainless steel carriers

Tests with 10^6 spores

		GROWTH BY DAY 5 IN Tryptic Soy Broth	
	Total Exposure time (minutes)	Nude spore carriers	Spore carriers wrapped and sealed in Tyvek® envelopes
Unexposed to Formalin Gas Plasma Sterilisation	0	9 / 9	9 / 9
Vaporised Formalin for 15 mins. + Gas Plasma for 0 mins. + ozone for 15 mins.	30	8 / 8	....
Vaporised Formalin for 15 mins. + Gas Plasma for 5 mins. + ozone for 15 mins.	35	8 / 8	
Vaporised Formalin for 15 mins. + Gas Plasma for 10 mins. + ozone for 15 mins.	40	8 / 8	
Vaporised Formalin for 15 mins. + Gas Plasma for 15 mins. + ozone for 15 mins.	45	8 / 8	....
Vapourised Formalin for 15 mins. + Gas Plasma for 18 mins. + ozone for 15 mins.	48	1 / 8	
Vapourised Formalin for 15 mins. + Gas Plasma for 20 mins. + ozone for 15 mins	50	3 / 8	
Vaporised Formalin for 15 mins. + Gas Plasma for 25 mins. + ozone for 15 mins.	55	0 / 8	5 / 8

Vaporised Formalin for 15 mins. + Gas Plasma for 30 mins. + ozone for 15 mins.	60	0 / 17	3 / 8
Vaporised Formalin for 15 mins. + Gas Plasma for 35 mins. + ozone for 15 mins.	65	0 / 8	0 / 8
Vaporised Formalin for 15 mins. + Gas Plasma for 40 mins. + ozone for 15 mins.	70	0 / 8	0 / 8
Vaporised Formalin for 15 mins. + Gas Plasma for 45 mins. + ozone for 15 mins.	75	.....	0 / 16
Vaporised Formalin for 15 mins. + Gas Plasma for 60 mins. + ozone for 15 mins.	90	.....	0 / 8 0 / 3*

* - Paper envelopes used for sterilisation wrapping instead of Tyvek(R), produced by Westfield Medical Ltd.

In order to meet the requirements of BS EN 556, a typical hospital load was selected. In this connection, and for the purpose of establishing process effectiveness, one load contained a high level of metals ("all metal load") and another load was made up of mixed materials, namely, metals, plastics and composites ("mixed load").

Eight spore carriers, each containing 10^6 spores, were mixed within each load of materials for testing in the gas plasma steriliser 10.

The "all metal load" contained hip prosthesis, spinal screws and plates, as well as arthroscopy shaving instruments and scalpels. The load was challenged with 8 x 10^6 *Bacillus stearothermophilus* stainless steel carriers and sealed within a Tyvek® bag.

A similar metal content was used for the "mixed load" adding a dialysis blood line kit, which contained a range of plastic materials as well as miscellaneous tubing found in hospital suction and infusion equipment. The mixed load was once again sealed inside a Tyvek® bag and challenged as per the "all metal load". Each load was treated by exposure to 15 minutes of formalin, 60 minutes of gas plasma/formalin mixture and 15 minutes of ozone.

Each experiment showed no growth of 8/8 carriers after 5-day incubation period in TSB media, whereas all controls grew on day one.

With 24/24 'no growth' results for each of the two types of load applied, it is evident that the process of the present invention is not susceptible to sterilisation failure due to metal shielding or other undesirable effects; even if any metal objects placed inside the chamber 11 are in direct contact with the chamber's inner walls.

In light of our investigations, we have found the following materials are all satisfactory for use with the process of the present invention.

Metals:

Stainless Steel (300 series), Brass, Aluminium (6000 series), Titanium

Plastics & Rubber:

Nylon, Latex Rubber, Polycarbonate, Polyethylene, Polypropylene, Teflon, PVC, Neoprene, Silicone, Delrin (black), Acrylics and Polystyrene

Other:

Glass, Glass Fibres, Ceramics, miscellaneous bonded materials using cyanoacrelates and UV-cure epoxies as the bonding agents.

In order to test the efficacy of the present process against vegetative bacteria, six types of vegetative bacteria (*Staphylococcus aureus*, MRSA, coagulase-negative staphylococci, *E. coli*, *Ps. aeruginosa* and *Salmonella typhimurium*) and the yeast, *Candida albicans*, were tested by preparing cultures at 10^6 / ml and then producing dried preparations on glass slides with at least 10^4 cfu's (colony forming units).

The slides were immediately exposed in batches of 8, with unexposed controls, to the process within the sterilisation chamber 11 for a total of 45 minutes (15 minutes formalin vaporisation, 15 minutes formalin/gas plasma sterilisation, 15 minutes ozone).

Immediately after sterilisation, the slides with bacteria were placed upside down on blood agar plates, and the slides with yeast were placed upside down on Sabouraud agar plates. The bacteria and yeasts grew as colonies on the agar under the glass slide. Plates were incubated for 24 hours at 37°C in air. Unexposed control cultures grew 10^4 cfu's.

With reference to the tabulated results (see Table 2 below) all six types of vegetative bacteria and *C. albicans*, each replicated 8 times, were killed by the sterilisation process of the present invention.

In addition, 56 out of 56 of the slides tested simultaneously in the gas plasma chamber 11 were killed thereof demonstrating the reliability and effectiveness of this process. Ozone alone has been tested against MRSA with some beneficial effect (Bennington and Pedler, 1998) but at a much lower concentration than used here in the third stage of the process (0.14 ppm instead of 35 ppm used by ourselves).

Table 2 Results of testing vegetative bacteria and fungi

Test results for unwrapped 'nude' 10^4 organisms on glass slides exposed to formalin vapour 15 minutes, formalin-gas plasma sterilisation for 15 minutes and ozone for 15 minutes (total exposure time of 45 minutes)

Pure cultures at 10^4 cfu	Failure to grow
Methicillin-resistant *Staphylococcus aureus* (MRSA)	8 / 8
Oxford *Staphylococcus aureus*	8 / 8
Coagulase-negative staphylococcus (CNS)	8 / 8
Escherichia coli	8 / 8
Pseudomonas aeruginosa	8 / 8
Salmonella typhimurium	8 / 8
Candida albicans	8 / 8
56 slides tested simultaneously in the sterilisation chamber	56 / 56

Secondly, each type of culture was mixed with 10^6 /ml *Candida albicans* to simulate a heavy organic load to investigate if the presence of the organic matter would seriously interfere with the formalin vapour-gas plasma sterilisation method as exhibited with phenolic disinfection of typhoid bacilli (Chick-Martin modification [1934] of the Rideal-Walker Test [1904]). Dried preparations of the mixtures of organisms, with a minimum of 10^4 cfu's of

each, were made on glass slides immediately before testing. Slides were exposed in the same manner as outlined above (15 minutes formalin, 15 minutes ozone) and then processed in the same manner as on the above.

As tabulated in Table 3 below, a minimal effect only, with survival of one to three colonies (cfu's) in two cultures out of 7 from a count of 10^4, was demonstrated with 15 minutes of formalin vapour-gas plasma. In this connection, and from the bacterial spore experiments above, it would be expected that this level of survival would be totally ablated by extending the period of gas plasma sterilisation from 15 to 30 minutes. These results show that the presence of a high load of organic matter had virtually no detrimental effect on the sterilisation process of the present invention.

Table 3 Results of testing vegetative bacteria and fungi with an organic load

Test results for unwrapped 'nude' 10^4 organisms mixed with 10^4 Candida albicans *on glass slides exposed to formalin vapour 15 minutes, formalin-gas plasma sterilisation for 15 minutes and ozone for 15 minutes (total exposure time of 45 minutes)*

Each culture at 10^4 mixed with *C. albicans* at 10^4	Failure to grow
Methicillin-resistant *Staphylococcus aureus* (MRSA)	8 / 8
Oxford *Staphylococcus aureus*	8 / 8
Coagulase-negative staphylococcus (CNS)	8 / 8
Escherichia coli	7 / 8*
Pseudomonas aeruginosa	8 / 8
Salmonella typhimurium	8 / 8
Mixture of all 6 bacteria above, each with 10^4 cfu, and *C. albicans* at 10^4 cfu	7 / 8**
* - 1 / 8 gave a growth of 3 colonies	
** - 1 / 8 gave a growth of 1 colony	

In addition to the above, batches of 8 seeded glass slides with 10^4 cfu's for each of the 7 pure cultures of organisms were wrapped and sealed in

Tyvek® envelopes and the experiment repeated in the manner described above.

As shown in Table 4, the gas plasma process fully penetrated through the sealed Tyvek® envelopes. In addition, there was total kill in two batches each of 28 slide tests conducted simultaneously, again demonstrating the reliability and effectiveness of this process.

Table 4 Results of testing vegetative bacteria and fungi

Test results for 10^4 organisms on glass slides wrapped and sealed in Tyvek(R) sterilising envelopes exposed to formalin vapour 15 minutes, formalin-gas plasma sterilisation for 60 minutes and ozone for 15 minutes (total exposure time of 90 minutes)

Pure cultures at 10^4 cfu sealed in Tyvek(R) envelopes	Failure to grow
Methicillin-resistant *Staphylococcus aureus* (MRSA)	8 / 8
Oxford *Staphylococcus aureus*	8 / 8
Coagulase-negative staphylococcus (CNS)	8 / 8
Escherichia coli	8 / 8
Pseudomonas aeruginosa	8 / 8
Salmonella typhimurium	8 / 8
Candida albicans	8 / 8
28 slides tested simultaneously in the sterilisation chamber in two batches	28 / 28 28 / 28

Testing for the effect of formalin-gas plasma sterilisation on mycobacteria was conducted with *M. chelonae*. Glass slides were prepared with 10^3 cfu's of *M. chelonae* and were exposed to sterilisation with formalin vapour for 15 minutes, formalin-gas plasma for 30 minutes and ozone for 15 minutes. Four controls were not exposed to sterilisation. All slides were inverted on to blood agar and incubated at 37°C for 7 days wrapped in plastic bags. The slides were then removed from each agar plate and the plates re-incubated for a further 7 days. This experiment was repeated for a further

8 slides containing 10^3 cfu's of *M. chelonae* but which were wrapped and sealed within Tyvek® sterilising envelopes.

Results were gained of 'no growth' for 8 out of the 8 slides seeded with *M. chelonae* both exposed 'nude' to formalin-gas plasma sterilisation, as well as wrapped and sealed within Tyvek® sterilising envelopes. Control slide cultures grew satisfactorily.

As regards the efficacy of the present process against viruses, we tested same against polio virus (vaccine strain type 2) and herpes simplex virus (type 2); each representative of the two major classes of viruses, namely, hydrophilic and lipophilic respectively. As will be appreciated, it was anticipated that the hydrophilic group would exhibit greater resistance to chemical sterilisation.

The polio virus was prepared in Reece monkey kidney cell lines with an approximate count of 10^3 infectious units. The herpes virus was prepared in human embryonic lung cell lines with an approximate count of 10^3 infectious units. Cell lines were incubated at 37°C. Viruses were freshly prepared for testing on dry glass coverslips, which were sealed in ampoules. There was a maximum delay of 8 hours between virus preparation and testing. Twelve replicates of each of the two viruses were produced for testing.

The sterilisation (GPS) process was applied as follows:

Six samples of the polio virus and six samples of the herpes virus ampoules were opened and immediately sealed within the Tyvek® sterilisation envelopes. These envelopes were placed in the sterilisation chamber 11 on two occasions.

The first experiment involved exposing 3 samples of polio virus and 3 of herpes to 15 minutes formalin, 30 minutes formalin-gas plasma and 15 minutes ozone.

The second experiment involved exposing 3 samples of polio virus and 3 samples of herpes to 15 minutes formalin, 60 minutes formalin-gas plasma and 15 minutes ozone.

Two samples of each virus were used for sterilisation (GPS) without wrapping in envelopes. Their ampoule cap was removed and they were placed directly in the sterilisation chamber 11.

One sample of each of polio virus and herpes virus were exposed to 15 minutes formalin, 30 minutes formalin-gas plasma and 15 minutes ozone.

One sample of each of polio virus and herpes virus were exposed to 15 minutes formalin, 60 minutes formalin-gas plasma and 15 minutes ozone.

Immediately after sterilisation the virus transport media was added and their tops resealed.

Four samples of polio virus and four samples of herpes virus were randomly selected from those produced for testing and defined as controls. These eight ampoules were opened and 5 drops of virus transport media added to each one. They were immediately resealed. This took place at the time that the other samples were being subjected to sterilisation by gas plasma.

Samples were collected from the laboratory and were sterilised 7 hours later. The untreated control ampoules were combined with the transport media at the commencement of the sterilisation process. All samples were returned to the laboratory in virus transport medium within 12 hours.

On return to the laboratory, the 24 samples were passaged in fresh tissue culture cell lines as described above. All 8 control samples gave cytopathogenic effects (CPE) in their respective cell lines within 2 days for the polio virus and within 4 days for the herpes virus. All 16 virus samples exposed to the differing periods of formalin vapour-gas plasma failed to give virus growth, recognised as CPE, in the tissue culture cell lines after 6 days incubation. Each cell line negative culture was passaged into a fresh cell line of the same type and further incubated for 6 days after which no sterilised (GPS) virus sample yielded any growth. This, we believe, demonstrates, the effectiveness of the present process in killing these two viruses.

As illustrated in Figures 2 and 3, a second embodiment of a sterilisation apparatus 10 in accordance with the present invention, which is portable, includes a wheeled housing 31 housing the working components thereof.

As illustrated such apparatus 10, can be connected to, or associated with, a room 11, which, in effect is the sterilisation chamber. That is, anything within the room 11 can be sterilised. Said room being provided with an inlet 36 and outlet 37 to which the working components of the sterilisation apparatus 10 can be attached.

Such apparatus 10 includes a chemical dosing unit 12, which, in use, can dispense the sterilising agent, for example, formalin or peracetic acid, in a gaseous state, via a hose or pipe 32, that is, by the action of pump 34, into the room 11 via inlet 36.

Within the room 11 there are plasma electrodes 17, which are attached to a plasma generator 13, via cables 35. Such plasma generator 13 includes an air inlet 14, an air pump 15 for drawing air into the plasma generator 13 and a HF transformer 16. It is to be understood that the room 11 or area to be

sterilised can be provided with the plasma electrodes 17 such that the sterilisation apparatus 10 can be simply connected thereto, or that a wall or walls of the room 11 are provided with an aperture through which the plasma electrodes 17 may protrude so that they are suitably located within the room 11. In any event, it is preferable that the plasma electrodes 17 are isolated and sealed such that they cannot come into direct contact with the items to be sterilised.

Air within the room 11 is re-circulated by air pump 21. That is, air is pumped into the room 11 via flexible pipe 32 and inlet 36 and is removed from the room via outlet 37 leading to flexible pipe 33.

The sterilisation apparatus 10, in this embodiment, is further provided with a gas analysing and monitoring support system 50, which, in use, can monitor the process parameters.

The housing further houses an ozone generator 40. Such generator 40 includes an air inlet 22, an air pump 23 and an ozone unit 24. In use, the air pump 23 draws dry air, preferably pure oxygen from a gas canister, into the generator 40 via the air inlet 22 and pumps the air through an air dryer and then through the ozone unit 24 and into the room 11 via flexible pipe 32 and inlet 36.

The sterilisation apparatus 10 is further provided with an extraction system including an activated carbon filter 20 and an air pump 21. In use, and after the generation of plasma, any harmful residue can be neutralised by extracting the gaseous contents of the room 11, via outlet 37 and flexible pipe 33, through the carbon filter 20.

A non-limiting example of the sterilising process in accordance with the present invention utilising the sterilising apparatus of Figures 2 and 3 will now be exemplified below:

A single occupancy hospital ward 11 was artificially seeded with MRSA (Staphylococcus Aureus (Oxford Strain)) with a 100,000 colony forming units (cfu).

The sterilising apparatus 10 was connected or linked to the ward 11. In this connection, the flexible pipes 33 and 32 were connected to an outlet 37 and inlet 36 provided in the wall of such ward 11 respectively.

The connector cables 35, which are connected to the gas plasma electrodes 17, located within the ward 11, were then connected to the plasma generator 13 of the sterilising apparatus 10. Due to the size of the ward 11 the gas plasma generator 13 was nearly three times the power of the first embodiment outlined above, for example, 600 watts.

Formalin gas at a concentration of 3000 ppm was then introduced from the chemical dosing unit 12 into the ward 11, that is, via pipe 32 and inlet 36.

The temperature, which was previously ambient, was increased from 22 °C to 27 °C, and the formalin gas was re-circulated via the re-circulation system 18 for 10 minutes within the ward 11.

The plasma generator 13 was then activated and left to run for 3 hours.

Neutralisation was effected by 30 minutes of ozone generation, which was fed into the ward 11 via flexible hose 32 connected to inlet 36.

There was then twenty air changes via the activated carbon filters 20 prior to re-entering the ward 11.

On entering the room, we collected all the randomly sited Staphylococcus and then incubated same for 24 hours. On doing so, no growth was recorded in any of the samples, except the control.

CLAIMS

1. A sterilisation process which is not carried out in a vacuum including the steps of:

introducing an aldehyde in a gaseous state into a sterilisation chamber or area to be sterilised; and

introducing gas plasma into the sterilisation chamber or area to be sterilised.

2. A sterilisation process as claimed in claim 1, wherein the aldehyde is formalin.

3. A sterilisation process as claimed in claim 2, wherein the sterilisation chamber or area to be sterilised is provided with formalin at a concentration of 2000-6000 parts per million (ppm).

4. A sterilisation process as claimed in claim 3, wherein the sterilisation chamber or area to be sterilised is provided with formalin at a concentration of 3000 ppm.

5. A sterilisation process as claimed in any one of claims 1 to 4, wherein the sterilisation process, subsequent to the introduction of plasma, further includes the step of introducing ozone into the sterilising chamber or area to be sterilised.

6. A sterilisation process as claimed in claim 5, wherein the sterilisation chamber or area to be sterilised is provided with ozone at a concentration of 80 ppm.

7. A sterilisation process as claimed in any one of the preceding claims, further including the step of neutralising any harmful residue by passing same through a carbon filter.

8. A sterilisation process as claimed in any one of the preceding claims, wherein prior to introducing plasma into the sterilisation chamber or area to be sterilised, the aldehyde is re-circulated within the sterilising chamber or area to be sterilised for a period of 5 to 15 minutes.

9. A sterilisation process as claimed in claim 8, wherein the aldehyde is re-circulated for a period of 10 minutes.

10. A sterilisation process as claimed in any one of the preceding claims, wherein gas plasma is introduced into the sterilisation chamber or area to be sterilised for 15-180 minutes.

11. A sterilisation process as claimed in claim 10, wherein the plasma is introduced for 45 minutes.

12. A sterilisation process as claimed in claim 10 or 11, wherein during the duration of the plasma producing stage, the gas within the sterilisation chamber or area to be sterilised is re-circulated.

13. A sterilisation process as claimed in any one of claims 10 to 12, wherein during the introduction of plasma the temperature within the sterilisation chamber or area to be sterilised is maintained from 25°C to 66°C.

14. A sterilisation process as claimed in 13, wherein during the introduction of plasma the temperature within the sterilisation chamber or area to be sterilised is maintained at 50°C or within +/- 3°C thereof.

15. A sterilisation process as claimed in any one of the preceding claims, wherein the temperature within the sterilisation chamber or area to be sterilised prior to the introduction or production of plasma is maintained from 22°C to 45°C.

16. A sterilisation process as claimed by any one of claims 1 to 15 substantially as hereinbefore described and exemplified.

17. A sterilisation apparatus when used to carry out the sterilisation process as claimed by any one of the preceding claims, the sterilisation apparatus being provided with a sterilisation chamber or being connectable to an area to be sterilised and including:

means for providing the sterilisation chamber or area to be sterilised with an aldehyde in a gaseous state; and

means for introducing or generating plasma within the sterilisation chamber or area to be sterilised.

18. A sterilisation apparatus as claimed in claim 17, wherein the sterilisation apparatus further includes means for re-circulating the air within the sterilisation chamber or area to be sterilised.

19. A sterilisation apparatus as claimed in claims 17 or 18, wherein the sterilisation apparatus further includes ozone producing means for introducing ozone into the sterilisation chamber or area to be sterilised.

20. A sterilisation apparatus as claimed in any one of claims 17 to 19, further including means for neutralising any harmful residue within the sterilisation chamber or area to be sterilised.

21. A sterilisation apparatus as claimed in claim 20, wherein the means for neutralising the harmful residue, subsequent to the production of plasma, include ozone producing means which dispense ozone into the sterilisation chamber or area to be sterilised.

22. A sterilisation apparatus as claimed in claim 19, wherein the ozone producing means can generate ozone at a rate of 1.5 – 1.9 grams per hour.

23. A sterilisation apparatus as claimed in claim 20, wherein the neutralisation means include a carbon filter through which air from the sterilisation chamber or area to be sterilised is drawn.

24. A sterilisation apparatus as claimed in claim 20, wherein the neutralisation means includes means for flushing the sterilisation chamber or area to be sterilised with clean air.

25. A sterilisation apparatus as claimed in claim 24, wherein the means for flushing the sterilisation chamber or area to be sterilised with clean air include a ULPA filter through which air from outside the sterilisation chamber or area to be sterilised is drawn.

26. A sterilisation apparatus as claimed in any one of claims 17 to 25, wherein the sterilisation apparatus is portable.

27. A sterilisation apparatus as claimed in claim 26, wherein the sterilisation apparatus is provided with wheels such that same can be wheeled from one location to another.

28. A sterilisation apparatus as claimed in any one of claims 17 to 27, wherein the power of the means for producing plasma ranges from 200 – 1200 watts.

29. A sterilisation apparatus as claimed in any one of claims 17 to 28, wherein the plasma generating means are isolated and/or sealed such that they cannot come into direct contact with the items to be sterilised.

30. A sterilisation apparatus as claimed in any one of claims 17 to 29 substantially as hereinbefore described with reference to the accompanying drawings.

31. A sterilisation process as claimed in any one of claims 1 to 15, wherein during the introduction of plasma, the pressure within the sterilisation chamber or area to be sterilised is maintained between 1 – 100, preferably 10 pascals.

INVESTOR IN PEOPLE

Application No: GB 0103237.4
Claims searched: 1 to 31

Examiner: Graham S. Lynch
Date of search: 30 August 2001

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.S): A5G (GAB)

Int Cl (Ed.7): A61L 2/14, 2/20; B65B 55/00, 55/02

Other: On-line : WPI, EPODOC, JAPIO

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
A	GB 2066076 A BIOPHYSICS RESEARCH. Whole document.	

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

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